

	PCR 25 µL reaction (units : uL)	Final Concentration		DNA		Primers
5X Q5 reaction Buffer	5	1X		CYC_3		f_Gbs_CYC
10 mM dNTPs	0,5	200 µM				r_Gbs_CYC
10 µM Forward Primer	1,25	0.5 µM				
10 µM Reverse Primer	1,25	0.5 µM				
DNA (1 ng/µL)	1	<1 ng				
DMSO (optional)	0	0				
Q5 high fidelity DNA Polymerase (added las	0,25	0.02 units/µl PCR				
Nuclease-free Water	15,75					
Total volume	25					
WORK ON ICE	CYC3					
number of reactions:	4					
Mastermix	volume in uL					
5X Q5 reaction Buffer	20		A	CYC3		
10 mM dNTPs	2		A-	CYC3 -		
10 µM Forward primer	5		B	CYC3		
10 µM Reverse primer	5		B-	CYC3-		
DMSO (optional)	0					
Q5 high fidelity DNA Polymerase (added las	1					
Nuclease-free Water	63					
Total volume	96					
Add 1µl DNA in tubes A and B						
Program A	CYC-Q5-A					
Initial Denaturation	98°C	30 seconds				
	98°C	10 seconds				
35 Cycles	66°C	30 seconds				
	72°C	30 seconds	Same temperat			
Final Extension	72°C	10 minutes				
Hold	4°C					
Program B	CYC-Q5-B					
Initial Denaturation	98°C	30 seconds				
	98°C	10 seconds				
	65°C	20 seconds				
35 Cycles	72°C	30 seconds				
Final Extension	72°C	5 minutes				
Hold	4°C					